OLFACTORY REACTIONS IN THE BRAIN
OF THE HEDGEHOG

By E. D. ADRIAN

From the Physiological Laboratory, Cambridge

THOUGH many parts of the brain have been studied by recording their electrical activity there have been few attempts to apply this method to the olfactory regions. One reason may be that smell is not an important sense in man; another is that in mammals the olfactory parts of the brain are covered by the neopallium and are therefore difficult to expose. The difficulty is not insuperable, however, and varies from one species to another. The present work arose from the observation that in the hedgehog the olfactory bulb and the pyriform lobe are large and easily accessible and show a well-defined electrical activity related to olfactory stimulation. The same kind of activity has also been found in the cat. In several respects the olfactory discharge in the mammal resembles that found by Adrian & Ludwig [1938] in the olfactory tract of fish, though there are differences due to the periodic character of the stimulus and to a greater tendency to synchronization in the discharge.

METHOD

Material. Diagrams of the brain of the hedgehog (*Erinaceus europaeus*) are given in Fig. 1. There is a description of the olfactory regions by Retzius [1897] and a full account of the anatomy and cell structure of a closely related brain in a paper by Gray [1924] on the opossum (*Didelphys virginiana*). It will be seen that in the hedgehog the neo-cortex does not overlap the olfactory part of the cerebrum as in most mammals, but forms an incomplete cap below which the pyriform lobe bulges laterally. This part of the lobe can easily be reached by an electrode after the skull has been opened from above and the olfactory bulb is also very easily accessible.   .

Electrodes. In the earliest experiments one electrode was earthed and was attached to the tissues at the side of the head to form an indifferent lead. But with this arrangement it was found that the large potentials

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developed in the pyriform lobe were bound to appear in the record what-ever the position of the other electrode on the brain. For better localiza-tion, therefore, two electrodes were usually placed a few millimetres apart on the region to be examined. For leading from the surface the electrodes were tufts of cotton-wool held in a spiral of silver wire coated with silver chloride. When the interior of the olfactory bulb was to be examined, an enamelled silver wire was used instead of one of the cotton-wool electrodes. Both were mounted on adjustable arms on a vulcanite plate fixed to the skull with sealing wax [cf. Adrian & Moruzzi, 1939]. The potentials were recorded in the usual way with a Matthews oscillograph and loud speaker.
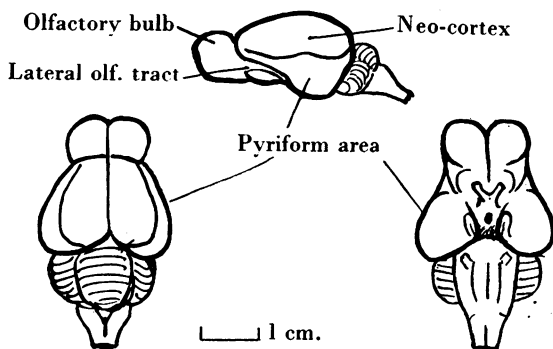

Fig. 1. Diagram of the brain of the hedgehog.

Anaesthetic. Most of the observations were made under nembutal anaesthesia of varying depth. Dial and chloralose were also used, the latter as an example of an anaesthetic with a different action from that of the barbiturates. Ether was unsatisfactory, as its use involved either the insertion of a tracheal cannula under another anaesthetic or else the exposure of the olfactory epithelium to ether vapour. Dial and nembutal certainly modify the activity of the olfactory pathways to some extent, but a comparison of different stages of anaesthesia shows that the modi-fication is only one of degree.

Olfactory stimulation. No attempt was made to use accurately measur-able olfactory stimuli, for the resulting activity cannot be measured accurately and there are too many uncertain factors, such as the amount of nasal secretion, the depth of anaesthesia and the rate of breathing. The method of stimulation originally employed was to place in front of the snout a small beaker containing an odorous solution or cotton-wool impregnated with an odorous substance. For stronger stimulation the

hedgehog was made to inspire through a wide glass tube which was fitted over the snout and lightly plugged with cotton-wool at the far end. A drop of tincture of asafoetida or oil of cloves could be placed on the cotton-wool at the appropriate moment. These simple methods were usually adequate, for with nembutal anaesthesia of medium depth reflex changes in breathing induced by the smell are rarely great enough to confuse the issue. But in the later experiments it was arranged that the air current through the nose should be independent of the depth of· breathing. This was secured by fitting a tracheal cannula through which respiration took place and passing a second cannula upwards through the larynx and into the back of the nose (Fig. 2). The cannula was connected
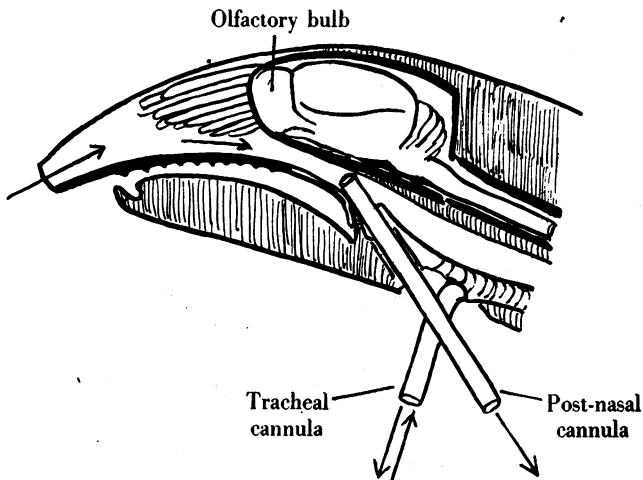

Fig. 2. Arrangement for drawing air through the nose independently of respiration.

with the suction side of a small respiration pump so that at each stroke a known volume of air was drawn through the nose. A minor trouble has been that in some animals the nasal secretions caused frequent blocking of the airway and consequent failure of stimulation unless the nose was cleared repeatedly.

RESULTS

Activity of the pyriform lobe

The response to air currents. The most prominent result is illustrated in the records in Figs. 3 and 4. These are made from animals under nembutal anaesthesia breathing quietly without any intentional olfactory stimulation. The periods of inspiration are marked above by a signal and it will be seen that at each inspiration there is a short group of potential

waves with a definite rhythm of 15–20 per sec. During the intervening period there are occasional waves at irregular intervals or there may be complete inactivity.
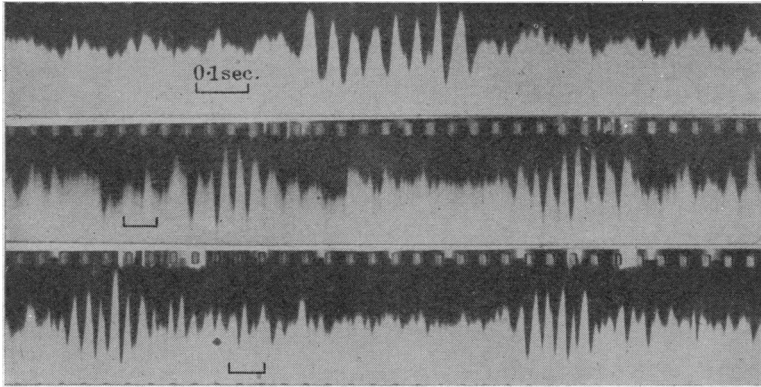

Fig. 3. Electrical activity of the pyriform lobe in animals breathing normal air. Records from three animals, all under nembutal anaesthesia. At each inspiration there is a series of regular potential waves at a frequency of 15–20 per sec. Horizontal line marks 0·1 sec. on each record. The large waves represent a change of 0·2 to 0·3 millivolts.
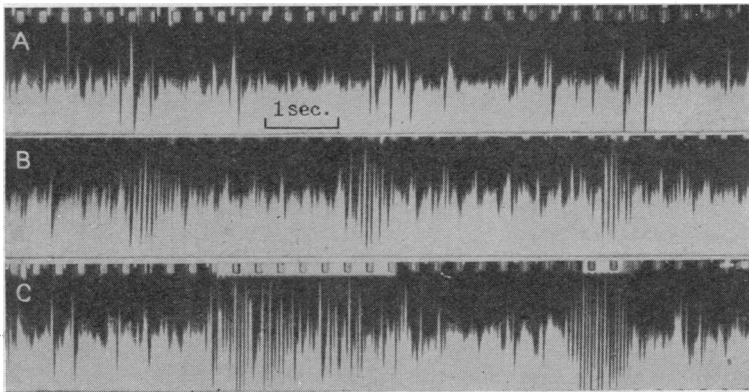

Fig. 4. Records from the left pyriform lobe in a hedgehog under nembutal. In A the left nostril was partially blocked with plasticene to reduce the flow of air through that side of the nose. In B the left nostril was free and the right was blocked. In C air was blown into the nostril from a glass syringe. Increasing the air current increases the number and frequency of the waves.

The regular wave groups are a true response of the olfactory system. They are not due to electrical spread from muscles or from parts of the brain directly concerned with respiration, for when the post-nasal

cannula is used the wave groups occur whenever air is sucked through the nose and bear no relation to the movements of breathing. If no air flows through the nose there are only occasional waves at longer intervals, and to produce the wave groups the air must flow through the nasal passages on the side under examination, for the groups are abolished by plugging the nostril on that side and increased by plugging the opposite side (Fig. 4 A, B).

But in spite of their olfactory origin the waves seem to depend more on the mechanical effect of the air current than on its smell. The velocity of the air through the nose is at any rate the principal factor in determining the rhythm. With very slow and shallow breathing there is some increase in the irregular waves during inspiration but no regular series; with deeper breathing the groups have a frequency which ranges from
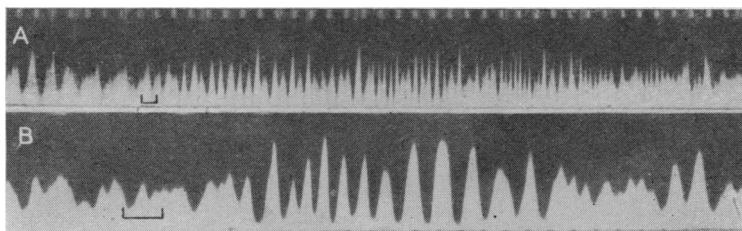

Fig. 5. Hedgehog under nembutal. Air sucked through the nose by post-nasal cannula arrangement (Fig. 2). In A the air current is increased gradually and the wave frequency rises. In B the current is reduced and the frequency falls. Horizontal line marks 0·1 sec. in this and all subsequent records.

15 per sec. in deep to 25 in light anaesthesia, and when air is blown or sucked forcibly through the nose the frequency may rise as high as 35–45 per sec. These changes in the response are illustrated in Figs. 4 and 5.

The degree of regularity and the range of frequency of the waves varies from one animal to another. In some there is only a slight rise of frequency when the air current is greatly increased; in others (usually under lighter anaesthesia) the maximum frequency which can be produced by blowing is about twice that of the normal rhythm. The records in Fig. 5 are enough to show that the frequency of the regular wave discharge can certainly be varied by changing the flow of air through the nose. In Fig. 5A for instance, a progressive increase in the air current makes the frequency rise from 16 to 30 per sec., and in Fig. 5B a decreasing air current gives a progressive slowing. But it is often impossible to obtain a smooth variation over a wide range of frequencies. One reason

for this is that the air ways through the nose may be blocked by fluid and are freed suddenly when the air pressure rises. The stimulus is then a sudden blast of air and the response is always near the maximal frequency. Another reason may be the anaesthetic, for under dial only the lower frequencies can be obtained.

The regular wave rhythms are produced by air which has no smell distinct enough to be appreciated by the human nose. The air of a laboratory cannot be regarded as free from smell and may be highly odorous to
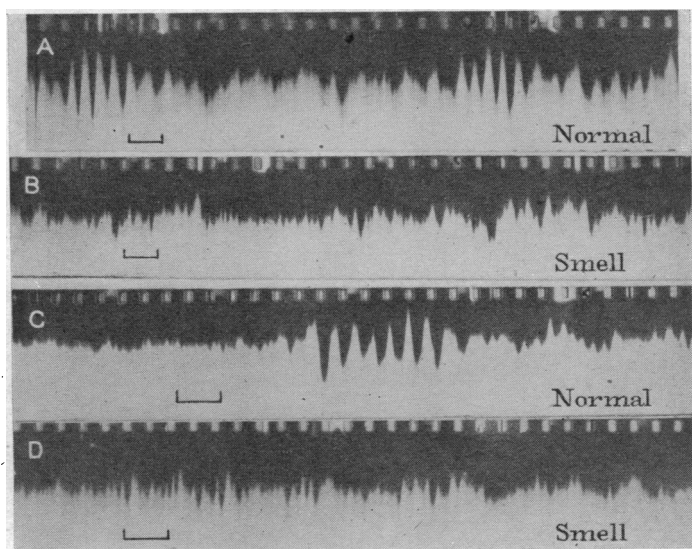

Fig. 6. The response to odours. Hedgehogs under nembutal. A and B from one animal. In A (normal air) regular waves appear in the pyriform lobes at each inspiration. In B the odour of asafoetida abolishes the waves. C and D from another animal; C breathing normal air, D breathing air with oil of cloves.

a macrosmatic animal; yet these regular responses seem to imply a uniform, synchronous activity which could scarcely be the basis for discriminative reactions to different smells. The way in which they are produced suggests instead that the waves represent a uniform mechanical stimulation by the air current and not a complex chemical stimulation by the odours in it. This view seems to be supported by the results of adding an odour strong enough to be detected by the human nose.

The response to odours. When a record is made from the surface of the pyriform lobe the result of adding a distinct odour to the air is to diminish or abolish the regular wave groups. In Fig. 6B for instance, a

drop of tincture of asafoetida was placed on cotton-wool close to the nose, and it will be seen that the wave groups no longer appear at each inspiration. In their place there are a few patches of small waves at a higher frequency, and it is only on removal of the cotton-wool that the large waves return. Similar results have been obtained with clove oil and decayed animal matter. In these records the animal was breathing
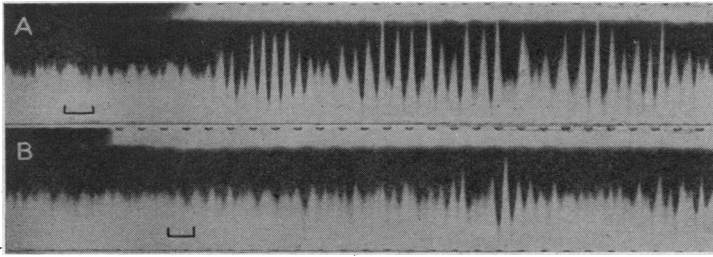

Fig. 7. The response to odours. Air drawn through the nose by post-nasal cannula. In A the air is normal, in B it contains the vapour of oil of cloves. The odour abolishes the large waves.
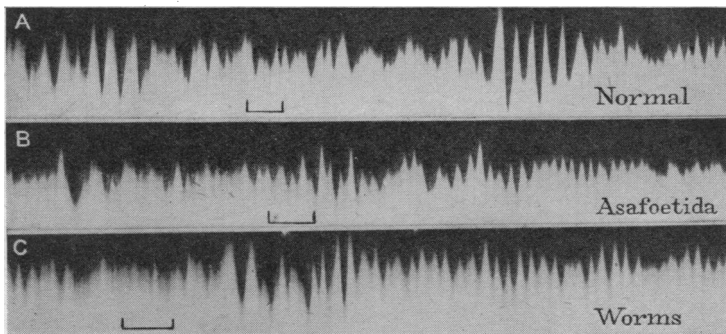

Fig. 8. Effect of intense odours. Hedgehog under nembutal. A, breathing normal air, waves 20 per sec. B, strong asafoetida, waves 52 per sec. C, odour of decayed worms, waves 48 per sec.

through the nose, and though there was no obvious change in the rate and depth of breathing it is conceivable that the abolition of the rhythm might have been due to a lessened air current. But the same result is given in experiments in which the air is sucked through the nose by the cannula arrangement shown in Fig. 2. A record illustrating the effect is given in Fig. 7. In this case the odour was that of oil of cloves, and there is the same disappearance of the regular waves.

If the smell is intense the large waves are abolished, but in their place there is a train of small rapid waves, more regular at each inspiration

but continuing throughout the whole respiratory cycle (Fig. 8). Here the slower synchronous wave response produced by the normal air current is converted into a rapid synchronous response by intense stimulation. It seems most likely, therefore, that the weaker stimulation causes an increase and not an inhibition of activity, and that the large waves are broken up because the different neurones are no longer all responding at the same frequency.

To decide the point we need to know whether there is in fact any increase in the discharge to the pyriform lobe when the large waves are abolished by an odour. It is not possible to record impulses in the non-medullated fibres of the olfactory nerve, but from certain parts of the olfactory bulb a wire electrode will pick up a characteristic activity and this gives most of the information we need.

Activity in the olfactory bulb

If an insulated silver wire is thrust into the olfactory bulb the crackling or rushing sound usually associated with a discharge of nerve impulses becomes audible when the wire has penetrated about 1·5 mm.
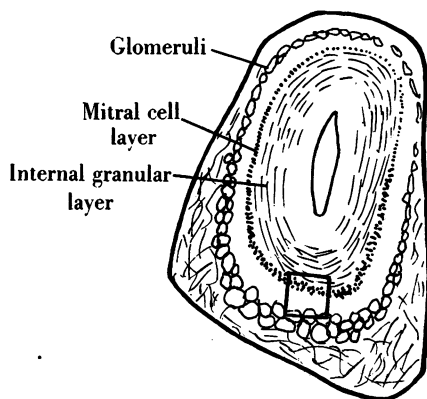

Fig. 9. Sketch of a section through the olfactory bulb to show the mitral cell layer and the region from which impulses can be recorded. The area stained by Hess's method is enclosed in a rectangle.

from the dorsal surface and again at a depth of about 5 mm. These two positions correspond with the layer of mitral cells and the axons which arise from them in the dorsal and ventral part of the bulb (Fig. 9). The localization was checked in one experiment by Hess's method [Hess, 1932]. An enamelled iron wire was used and when the active layer

was reached a current was passed through the wire to produce a deposit of iron in the tissues and this was afterwards stained with ferricyanide.

By manipulating the wire it is usually possible to obtain a record in which the potential changes are mostly brief axon spikes, though the amount of activity has always been too large to allow much inference as to the discharge in each unit. The activity consists of isolated bursts of impulses (or rather of potential spikes) between breaths with a more continuous discharge at each inspiration: and there is an immediate increase in the discharge if asafoetida or clove oil is brought near the nose.

The isolated bursts of impulses occur whenever there is a fairly long pause between each breath and the next. They are spaced rather irregularly at a frequency of 2–5 per sec. and they continue as long as the olfactory organ is intact. From some parts of the bulb indeed these irregular bursts are all that can be picked up, although from other parts there is a definite inspiratory effect as well. The bursts evidently correspond with the irregular waves recorded from the pyriform lobe and may be regarded as a resting discharge like that from other slowly adapting sense organs. Like other resting discharges they disappear for a few seconds after a period of increased activity, and for this reason they are absent when the breathing is moderately rapid and deep. It may be recalled that a considerable resting discharge was found by Adrian & Ludwig in the olfactory tract of the catfish. This also is abolished for a time following a period of increased activity.

When normal air (without intentional smell) is breathed the resting discharge quickens at each inspiration into a succession of outbursts at 15–20 per sec. (Fig. 10). These are evidently responsible for the regular waves in the pyriform lobe. When air is blown into the nostril or sucked through it by the post-nasal cannula the impulses are more definitely grouped into a series of volleys with a frequency of 30–40 per sec. Here also there are the regular waves in the pyriform lobe to correspond.

When clove oil or asafoetida is added to the air there is always an increase in the noise of the discharge at inspiration, and with a strong smell the increase may start before an inspiration has occurred. But in every case unless the smell is intense it has given a continuous stream of impulses rather than synchronised outbursts. Towards the end of the active period a grouping may appear again at a higher frequency if the smell is strong. Records illustrating the change from the grouped discharge of normal breathing are given in Fig. 11. They show that the disappearance of the regular wave response of the pyriform lobe is

not due to any lack of activity. With the specific olfactory stimulus the discharge from the olfactory bulb increases, but the increase is accompanied by a failure of the synchronization which was present when the air had no distinct smell.
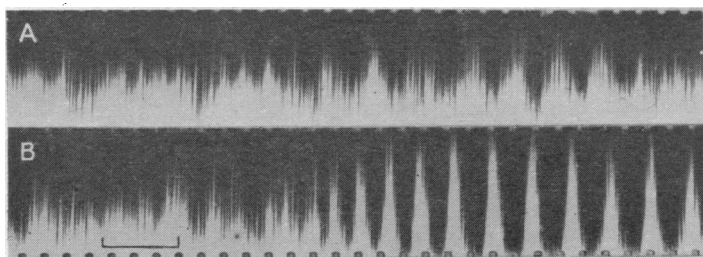

Fig. 10. Records from the mitral cell layer and fibres of the olfactory bulb. Hedgehog under nembutal. A, normal breathing. The bursts of the resting discharge quicken at inspiration to the 15 per sec. rhythm. B, blowing air into the nose. Synchronized volleys with an initial frequency of 30, falling to 20 per sec.
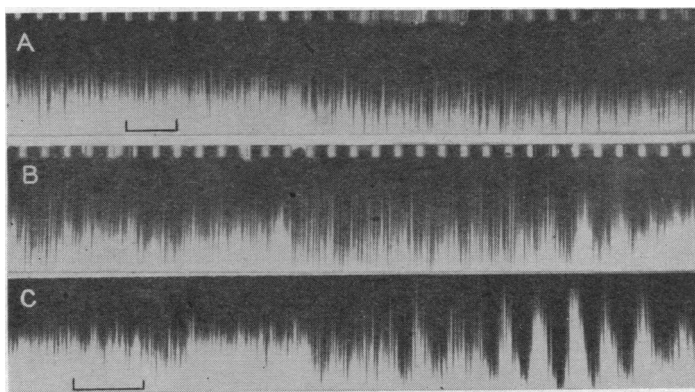

Fig. 11. Same animal as Fig. 10. Effect of adding a distinct odour. All three records show the effect of asafoetida. In A the discharge at inspiration is increased but there is no synchronization. In B and C synchronization begins towards the end of the discharge.

Substances causing an olfactory discharge

No attempt has been made to test a large number of odours. The substances regularly used were oil of cloves and tincture of asafoetida and these were always effective. Tincture of valerian, camphor and phenol were used occasionally and responses have been obtained with all three. Water in which an earthworm had been allowed to rot usually gave a large discharge when it was brought near the nose, but there was some-

times an inhibition of breathing to complicate the result. In all probability a smell with a weakly stimulating effect would not produce an obvious breakdown of the synchronized rhythm and would not cause any marked alteration in the olfactory bulb discharge unless the electrode happened to be favourably placed. Thus the method at present could scarcely be trusted to reveal threshold effects.

A point of some interest was the intense stimulation produced by tobacco smoke. If a small beaker is held in front of the nose and filled with cigarette smoke the discharge is much greater than that produced by asafoetida or clove oil and very soon becomes a regular series of volleys at a frequency of about 40 per sec. (cf. Fig. 12). This happens in an animal
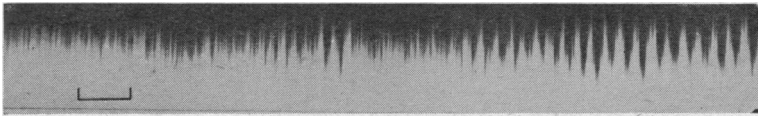

Fig. 12. Intense discharge produced by cigarette smoke. Hedgehog under chloralose with wire electrode in the olfactory bulb. Diffusion of smoke into the nose starts a series of volleys at 40 per sec.

breathing naturally although the synchronized discharge resembles that produced by blowing air forcibly through the nose; in some animals, moreover, the volleys may develop and persist although respiratory movements are completely inhibited. Thus an air current through the nose is not the only form of stimulus to give a synchronized discharge.

Olfactory responses in the cat's brain

Although only five successful experiments have been made on cats the results are enough to demonstrate that the olfactory response is of the same general character as in the hedgehog. To reach the lateral olfactory tract and the pyriform lobe, the eyeball was removed so as to expose the posterior wall of the orbit; a trephine opening over the frontal area was then enlarged downwards until the tract came into view. A cotton wool electrode resting on the tract will then pick up regular waves at each inspiration and the noise of impulses can often be detected by the loud speaker. The waves are presumably derived from the cortex of the pyriform lobe for they can be detected over a wide area whereas the impulse noise is confined to the tract. In three cats under nembutal the waves at inspiration had a frequency covering much the same range as in the hedgehog—from 12 per sec. in quiet breathing to 35 per sec. when air is blown into the nose. Under dial (two experiments) the frequency

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has shown less variation. Asafoetida and clove oil have produced a considerable increase in the noise of the impulse discharge, but their effect on the regular waves is uncertain, for changes in the rate and depth of breathing have always occurred. Records from the cat's brain are given in Fig. 13. The resemblance to the hedgehog response is unmistakable, though more data will be needed for an accurate comparison.
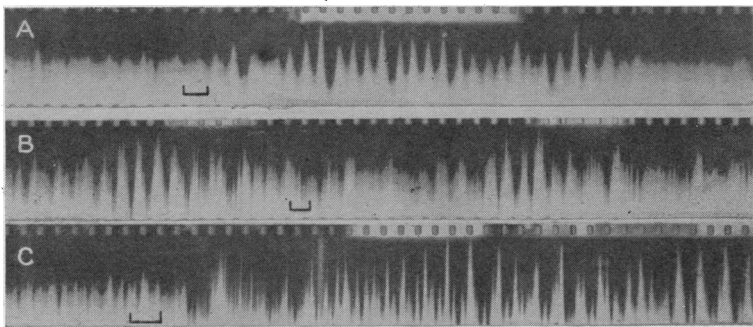

Fig. 13. Records from cats. Electrode on the surface of the lateral olfactory tract. A, cat under dial. Regular waves at 15 per sec. during normal inspiration. B, cat under nembutal. Large waves at 10 per sec. during inspiration. C, same animal as B. Increased activity due to cigarette smoke, maximum frequency 40 per sec.

DISCUSSION

It is interesting to find that even in deep anaesthesia there is a periodic activity in the olfactory part of the brain in phase with respiration. It was thought that it might perhaps be an important factor in maintaining the general level of excitation of the cerebrum, and that it might account for the periodic wave trains which appear in other parts of the anaesthetized brain. But in a cat under deep dial the waves which appear from time to time in, for example, the suprasylvian gyrus seem to be quite unaffected by procedures which would cause great modifications in the olfactory activity. Thus blocking the nose or blowing down it does not reduce or increase the periodic activity in the neo-cortex. In light anaesthesia these procedures certainly produce a widespread effect on the cortical waves, but clearly in deep anaesthesia the olfactory discharge has little to do with the periodic waves which occur in the non-olfactory areas.

Apart from this the main finding has been that an air current through the nose gives a response in the olfactory area made up of large regular waves, and that a smell converts this into small irregular waves. It is true that this statement needs some qualification; for instance, a rapid

air current will give large regular waves at a high frequency whether the air has a distinct smell or not, and an intense smell will give a regular high-frequency response on its own account. But there remains the point that over a considerable range an increase in the air current and an increase in the concentration of the odour have different effects, as though the air current tended to promote and the odour to prevent a synchronized discharge. To account for this it has been suggested that the olfactory receptors can be stimulated mechanically as well as chemically, and that the mechanical stimulation is more likely to be uniform.

The chief uncertainty arises from the fact that in these experiments the air passing through the nose could never be regarded as completely odourless. The regular waves found with normal breathing might therefore have been due to a weak chemical stimulation of the olfactory organ, the response becoming synchronized because with threshold stimuli the lowest frequencies would predominate. An explanation on these lines is scarcely possible, however, for it does not explain why an increase in the air current alone has never produced an irregular activity like that due to the addition of an odour. Instead it has given a regular response at a higher frequency. Another reason against the explanation is that the response to an air current is not modified in any way by the odours in it unless these are fairly strong. No doubt there is some olfactory stimulation by the odours from rubber tubes, from the operation wound or from the animal's own nose; none the less the waves in the pyriform lobe have been just as large when steps have been taken to reduce these odours as far as possible. Air which has been collected from the roof of the building and stored in clean glass vessels with no rubber connexions seems to have just the same effect when injected into the nose as air from the workroom. This absence of any obvious effect from weak odours is difficult to explain if the regular waves are set up by chemical stimulation; it is intelligible if we suppose that the effects of the weak odours are swamped by those of the air current acting as a mechanical stimulus.

It might be objected that we have no evidence from our own sensations that the olfactory organ can be stimulated by the movement of air over it. We do know, however, that the olfactory organ of the catfish responds to mechanical stimuli, for it responds to light pressure and to a current of water containing inert particles in suspension [Adrian & Ludwig, 1938]. Pressure on the olfactory organ has given a discharge in the hedgehog also, though it may have caused permanent injury. It is conceivable, therefore, that an air current might stimulate by bending

the hairs of the receptor cells, for it would be likely to cause some displacement in the layer of fluid and mucus covering the epithelium.

For the present then the view that there is normally a mechanical as well as a chemical stimulation of the olfactory organ seems to offer a reasonable explanation of the facts. In any case the facts leave no doubt that the excitation caused by a smell of moderate intensity is unevenly distributed and so tends to prevent a synchronous response; and from this it is a small step to the suggestion that the unevenness, i.e. the pattern of the excitation, will be different for different smells. It must be admitted that the present experiments give no direct proof that this is so. But their results are enough to support a comparison of the olfactory system with the visual. With both an intense stimulus gives a synchronized response at a high frequency, and with both a low-frequency rhythm, if it is present, is abolished by the kind of stimulus which leads to a discriminative reaction. A visual pattern abolishes the 'dark' rhythm of the eye of *Dytiscus*, or the resting rhythm of the striate area, because it forces the different units to respond at different frequencies, and a smell of moderate intensity abolishes the low-frequency response to an air current for the same reason. We recognize a sight not because particular receptors are stimulated but because a particular pattern of activity is aroused, and it is reasonable to conclude that we recognize a smell in the same way.

For a smell to produce a specific pattern of excitation in the olfactory epithelium we need only suppose that the different receptors are not all equally sensitive to different chemical stimuli. Such a differential sensitivity might depend on the intrinsic properties of the receptors, and it might also be due to extrinsic factors such as the amount of mucus in different regions, the rate of diffusion of the active molecules, etc. In this way an endless variety of smells might be distinguished because the process would be comparable not to the discrimination of colours but to that of visual patterns. It is to be hoped that further experiments will show whether this view can be confirmed.

CONCLUSIONS

1. In the hedgehog the olfactory bulb and the olfactory area of the brain are easily exposed and show a characteristic electrical activity in nembutal or chloralose anaesthesia.

2. Normal breathing produces a regular series of large potential waves in the pyriform area at each inspiration. These waves are due to the passage of air through the nose on that side. Their frequency varies from

15 per sec. during quiet breathing to 45 per sec. if the air is blown or sucked forcibly through the nose.

3.' If a distinct odour (clove oil or asafoetida) is added to the air, the regular waves no longer appear at inspiration and their place is taken by small irregular waves. An intense smell may produce a continuous series of small waves at a high frequency (50 per sec.).

4. If a wire electrode leads from the mitral cell layer of the olfactory bulb, a discharge of impulses can be heard at each inspiration. The addition of a distinct odour increases the discharge. When air is blown through the nose the impulses become grouped into volleys at a high frequency.

5. These results are best explained on the assumption that the olfactory organ can be stimulated mechanically by the air current as well as chemically by the odours in it. The air current produces a uniform excitation which results in synchronous waves at a high or low frequency The chemical stimulation is not uniform and so hinders the development of synchronous waves.

6. It is suggested that different chemical stimuli produce different distributions of excitation and that a familiar smell, like a familiar sight, is recognized by the specific pattern which it arouses in the brain.

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